TRAINING UPDATE

Lab Location:	GEC, SGMC & WAH	**Date Distributed:**	5/5/2017
Department:	Core Lab	**Due Date:**	5/24/2017
		Implementation:	**5/24/2017**

DESCRIPTION OF PROCEDURE REVISION

Name of procedure:

Prothrombin Time (PT) and INR SGAH.G03 v6

These apply to SG & WAH only:

Fibrinogen SGAH.G05 v7

D-Dimer SGAH.G04 v7

All will now be system SOPs

Description of change(s):

QC frequency changed to match practice / be less restrictive.
Most other changes are format updates

Section	Reason
4, 6	Remove individual section labeling instructions and add general one
6.3	Remove run QC at beginning of shift
8.3	Specify 5 minute soak with stirring for decontamination of stir bar ***(applies to PT SOP only)***
10.5	Review data moved from section 6
15	Update to new standard wording

Notes:

- Only the PT & Fibrinogen SOPs are attached.
- PTT SOP will be updated upon approval of critical value change (more info to follow)

These revised SOPs will be implemented on May 24, 2017

Document your compliance with this training update by taking the quiz in the MTS system.

Technical SOP

Title	**Prothrombin Time (PT) and INR**	
Prepared by	Ashkan Chini	Date: 3/10/2011
Owner	Robert SanLuis	Date: 6/3/2014

Laboratory Approval	**Local Effective Date:**	
Print Name and Title	**Signature**	**Date**
Refer to the electronic signature page for approval and approval dates.		

Review		
Print Name	**Signature**	**Date**

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TABLE OF CONTENTS

1. TEST INFORMATION

Assay	Method/Instrument	Local Code
Prothrombin Time / INR	Clot based assay / STA® Compact	PTA

Synonyms/Abbreviations
Prothrombin, PT/INR

Department
Coagulation

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2. ANALYTICAL PRINCIPLE

STA®-Neoplastine CI PLUS is used for Prothrombin times, and extrinsic Factor Assays on the STA ® Compact. A mixture of thromboplastin is added to citrated plasma and the time of clot formation is determined. The STA® Compact is a fully automated coagulation instrument, which uses an electromagnetic mechanical clot detection system. The oscillation of a steel ball within the cuvette with the thromboplastin and plasma is monitored by the STA® Compact. When the oscillation of the steel ball is stopped, by clot formation, the sensor registers the time in seconds. The prothrombin time is a basic coagulation-screening test for the assessment of congenital and acquired deficiencies of the extrinsic pathway (factors II, V, VII, X). The prothrombin time can be prolonged in certain clinical states, i.e. warfarin therapy, intestinal reabsorption disorders, liver failure, fibrinolysis and DIC.

The prothrombin time is also used to monitor warfarin therapy because of its sensitivity to variations in the concentration of the Vitamin-K dependent factors II, VII and X. Because of the variations in the prothrombin time results with different thromboplastins and instruments, it is recommended that the prothrombin time results be converted to an INR. The INR corresponds to the value of the ratio of the patient's PT and the geometric mean PT of the normal reference population raised to the ISI (International Sensitivity Index) power:

$$\text{INR} = \left(\frac{Patient's\ PT}{Geometric\ Mean\ PT} \right)^{\text{ISI}}$$

The ISI value of a given thromboplastin is determined by performing PT's on normal plasmas and coumadin-treated patient plasmas with the given thromboplastin and the WHO (World Health Organization) reference thromboplastin. The slope of this regression curve of the matched pairs is the ISI for the thromboplastin. The ISI of the WHO reference thromboplastin is 1.0.

3. SPECIMEN REQUIREMENTS

3.1 Patient Preparation

Component	Special Notations
Fasting/Special Diets	N/A
Specimen Collection and/or Timing	Normal procedures for collecting plasma may be used for samples to be analyzed by this method. Vacutainer tube must be filled to the line to ensure the proper ratio of blood to anticoagulant.
Special Collection Procedures	If hematocrit >55%, refer to appendices A and B for collection instructions.
Other	N/A

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3.2 Specimen Type & Handling

Criteria	
Type -Preferred **-Other Acceptable**	PLT Poor Plasma (sodium citrate) None
Collection Container	Light blue top tube (3.2% sodium citrate) Citrated blood 9:1 (blood to anticoagulant)
Volume - Optimum **- Minimum** **- Optimum** **- Minimum**	2.7 mL (9:1 blood to anticoagulant) in a 2.7 ml tube 2.4 mL (9:1 blood to anticoagulant) in a 2.7 ml tube 1.8 mL (9:1 blood to anticoagulant) in a 1.8 mL tube 1.8 mL (9:1 blood to anticoagulant) in a 1.8 mL tube
Transport Container and Temperature	Light blue vacutainer (as above) or a clean plastic screw capped vial at room temperature.
Stability & Storage Requirements	Room Temperature: 24 hours
	Refrigerated: Not recommended
	Frozen plasma: 2 weeks (PLT Poor Plasma) -20C or colder
Specimen preparation	Centrifuge whole blood for specified time /speed documented on each centrifuge for preparing platelet-poor plasma.
Unacceptable Specimens & Actions to Take	Specimens that are unlabeled, improperly labeled, or those that do not meet the stated criteria are unacceptable. Clotted, under-filled or over-filled tubes are not accepted. Request a recollection and credit the test with the appropriate LIS English text code for "test not performed" message.
Compromising Physical Characteristics	Moderate to gross hemolysis. Reject sample and request a recollection. Credit the test with appropriate LIS English text code HMM (Specimen moderately hemolyzed) or HMT (Specimen markedly hemolyzed) Lipemia: Acceptable Icterus: Acceptable
Other Considerations	Samples for unfractionated heparin testing should be centrifuged within one (1) hour from the time of specimen collection.

NOTE: Labeling requirements for all reagents, calibrators and controls include: (1) Open date, (2) Substance name, (3) Lot number, (4) Date of preparation, (5) Expiration date, (6) Initials of tech, and (7) Any special storage instructions. Check all for visible signs of degradation.

4. REAGENTS

The package insert for a new lot of kits must be reviewed for any changes before the kit is used. A current Package Insert is included as a Related Document.

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4.1 Reagent Summary

Reagents	Supplier & Catalog Number
NEOPLASTINE® CI Plus	Diagnostic Stago (REF 00667)
Pure Reagent Grade water	Millipore or NERL Thermo Scientific (Cat. No. 0015)

4.2 Reagent Preparations and Storage

Kit Component contains reagent 1 & 2	
Reagent 1	**STA® Neoplastine CI Plus**
Storage	2-8°C
Stability	Stable until expiration date on the vial. Once reconstituted: • It remains stable in its original capped vial without the stirring-bar, STA®-Reducer, for 8 days at 2-8°C. • It remains stable with the stirring-bar, STA®-Reducer and perforated plastic cap in place, for 48 hours on STA® Compact.
Preparation	Transfer the entire contents of one vial of Reagent 2 into one vial of Reagent 1 of the same lot. Allow the reconstituted reagent to stand at room temperature (18-25°C) for 30 minutes. Swirl gently. Add a stirring bar* to the vial and place the STA® Reducer and install the perforated plastic caps. Request the product drawer to open through MAIN MENU under LOADING and bar code the reagent. Place the reagent into a stirring position in the product drawer on the STA® Compact.

* Decontaminate stir bars once per week. Refer to section 8.3 for procedure.

Reagent 2	**10 ml Solvent**
Container	Manufacturer supplied vial.
Storage	2-8°C
Stability	Stable until expiration date indicated on the box label.
Preparation	Ready to use.

Reagent 3	**NERL Reagent Grade water**
Container	Manufacturer supplied vial
Storage	Room temperature
Stability	Stable 30 days after opening.
Preparation	Ready to use

5. CALIBRATORS/STANDARDS

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5.1 Calibration Procedure

The pre-calibrated PT values are identical for all the vials of each lot.
To enter the calibration data on the analyzer, scan the barcode printed on the assay value insert across the instrument barcode reader.
The calibration data will be validated for the lot being used once the Stago PT controls are run and tested.

The calibration curve is considered verified for the new reagent lot when both the STA®-Coag Control N + ABN Plus are within acceptable range. The acceptable STA®-Coag Control N + ABN Plus range is supplied by Stago. QC ranges must fall within the acceptable range which is established utilizing the peer group data in combination with our current/historic analytic performance.

6. QUALITY CONTROL

6.1 Controls Used

Controls	Supplier and Catalogue Number
STA - Coag Control N + ABN Plus	Stago Diagnostics, Cat. No. 00677

6.2 Control Preparations and Storage

Control	**STA - Coag Control N + ABN Plus**
Preparation	Reconstitute each vial of Reagent 1 or 2 with exactly 2 mL of reagent grade water. Allow the reconstituted material to stand at room temperature (18 - 25°C) for 30 minutes. Then, mix by turning the vial upside down, 3 – 4 times, to obtain a homogeneous solution.
Storage	2 - 8°C
Stability	The reagents in intact vials are stable until the expiration date indicated on the box label, when stored at 2 - 8°C. Once reconstituted, Reagents 1 and 2 remain stable in their original vials for 24 hours on STA Compact.

1. QC can be run automatically at pre-set intervals (in Test Set-up) or by ordering manually from the Quality Control Menu.
2. All control ranges are monitored automatically by the STA® Compact. If any controls are outside the ± 2 SD range, the instrument will audibly and visually alarm the operator. Otherwise, the results can be found in the individual QC files. Control results are automatically filed in the STA® Compact QC file. All results for a 24-hour period are converted to a "mean" value at midnight. This mean is

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used in the statistical data and is plotted on the Levy-Jennings chart as a daily mean.

3. To print all the QC data points for the PT test, perform the following procedure prior to midnight. From the MAIN MENU under CAL. /CONTROL select QUALITY CONTROL press **Enter** ↵. Cursor to the PT test and press **Enter** ↵to view the Levy-Jennings chart. Press **F1** to view the results in tabular form. Press **F6, select Execute then** press **Enter** ↵to print the individual values under current controls. Press ESC key to exit (back to graph). Press **F2** or **F3** to view other levels and continue with **F1** to view the result list.

6.3 Frequency

Controls are run ~~*at the beginning of each shift and*~~ every 4 hours of patient testing and with the change of any reagent used in test performance.

Controls are run after any maintenance is performed on the analyzer.

6.4 Tolerance Limits and Criteria for Acceptable QC

Step	Action
1	The established QC ranges are in the QC file of the STA Compact. The quality control results from the instrument are transmitted to Unity Real Time and can be viewed in that program. Any out-of-range QC results will be flagged.
2	If all controls are within QC parameters all sample results can be reported.
3	Rejected runs must be effectively addressed by corrective action. Steps taken in response to QC failures must be documented. Patient samples in failed analytical runs must be reanalyzed. Supervisor may override rejection of partial or complete runs only with detailed documentation that follows criteria that is approved by the Medical Director.
4	Corrective action documentation must include the following: QC rule(s) violated, the root cause of the problem, steps taken to correct the problem, how patient samples were handled, and the date and initials of the person recording the information. See the QC/QA SOP "QC Responsibilities" for more detail.
5	If the assay is down and results will not be reported in the scheduled turnaround time, clients will be notified of the situation.

6.5 Documentation

- QC tolerance limits are programmed into the instrument and Unity Real Time; it calculates cumulative mean, SD and CV and stores all information for easy retrieval.

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- Quality control records are reviewed daily at the bench, weekly by the Group Lead or designee, and monthly by the Supervisor/Manager or designee.
- Refer to complete policies and procedures for QC documentation and for record retention requirements in the Laboratory QC Program.

6.6 Quality Assurance Program

- Each new lot number of reagent or new shipment of the same lot of reagent must be tested with external control materials and previously analyzed samples. Performance of the new lot must be equivalent to the previous lot; utilize published TEA for acceptability criteria.
- Training must be successfully completed and documented prior to performing this test. This procedure must be incorporated into the departmental competency assessment program.
- The laboratory participates in CAP proficiency testing. All proficiency testing materials must be treated in the same manner as patient samples.
- Monthly QC must be presented to the Medical Director or designee for review and signature.
- Consult the Laboratory QC Program for complete details.

7. EQUIPMENT and SUPPLIES

7.1 Assay Platform

STA® Compact – Analyzer

7.2 Equipment

- Refrigerator capable of sustaining 2–8°C.
- Freezer capable of sustaining range not to exceed -20 to -70°C.
- Centrifuge calibrated for preparing platelet-poor plasma

7.3 Supplies

- Magnetic Stir Bars
- Cuvette Roll – Diagnostic Stago
- STA – brass adaptors
- STA – Reducer
- STA - Cleaner solution
- Plastic micro cups
- Plastic transfer pipettes

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8. PROCEDURE

NOTE: For all procedures involving specimens, buttoned lab coats, gloves, and face protection are required minimum personal protective equipment. Report all accidents to your supervisor.

8.1	Instrument Set-up Protocol
1	At the start of each shift, verify instrument temperatures and availability of cuvettes and cleaner solution by accessing the System Status screen from the main bar.
2	Verify instrument temperatures every shift and record on the maintenance sheet. If the Needle number 3, measuring block, or reagent drawer temperatures are out of range, corrective action must be taken prior to patient samples being run.
3	Make sure that there is an adequate supply of reagents in the analyzer, and they are in date.
4	Load cuvettes and cleaner/wash solution on the analyzer if needed.

8.2	Analytical Procedure
1	Refer to START-UP procedure for STA® Compact before running patient specimens on the STA® Compact at the start of each shift.
2	Request quality control. Through MAIN MENU under CALIB. /CONTROL select QUALITY CONTROL and press **Enter** ↵. Cursor to the PT (or PT+) test. Select PT (or PT+) by pressing **F1** and then **F10.** Type in your Access Code to run the QC.
3	Load patients' samples: Access the sample drawer(s) through the MAIN MENU, under LOADING, Select Sample, press **Enter** ↵. After the drawer opens, identify the type of specimen, such as micro sample (press **F8**), or stat (press **F12**). Identify the sample by bar coding or manually entering on the keyboard the patient identification number and then placing the specimen into the drawer.
4	In MANUAL MODE, the operator must order the test(s) from the Selection menu or from the Recorded Profile/s Cursor to the test and press **Enter** ↵to select. When all tests are ordered, press F10 to save.
5	In AUTO MODE, the STA®/STA® Compact will automatically order the test(s) selected in the AUTO MODE profile.
6	If TELELOADING is selected as the AUTO MODE profile, the STA®/STA® Compact will query the host computer and download the test(s) as well as assign the status (i.e. stat).
7	As soon as the sample drawer closes, the TEST STATUS screen will appear. If there is not enough reagent(s) to run the test(s), the suspect reagent(s) will appear in red with the amount of depletion. This depletion of reagent will BLOCK the SAMPLE PIPETTING. When this occurs, add the necessary reagent(s) to run the samples by responding N (NO) to the warning message 'NEW TESTS ARE DELAYED - REACTIVATE?' Reagents can then be loaded in the drawer. By responding Y (YES) to the warning message 'NEW TESTS ARE DELAYED - REACTIVATE?', the instrument will continue to perform all tests for which there is sufficient reagent (i.e.

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8.2	Analytical Procedure
	while waiting for reagents to stabilize after reconstitution?
8	All patient results are displayed on the TEST PANEL screen and automatically print out and transmit if selected on the system status menu.
9	For results in question that need operator intervention, cursor to the identification number in the TEST PANEL screen and press enter. This will display the FILE PROCESSING screen. Follow the options on the left-hand side of the screen (i.e. **F3** - rerun test).

8.3	Stir Bar Decontamination
1	Immerse the bars in a vial of Desorb and let them soak for a five (5) minutes with constant magnetic stirring.
2	Transfer the bars from the Desorb vial to a vial of Reagent Grade Water and let them soak for ~~a few~~ another five (5) minutes with constant magnetic stirring. Repeat this rinsing step with another vial of Reagent Grade Water.
3	Rinse the bars with Reagent Grade Water and dry them carefully to remove all traces of moisture before adding them to reagent vials.

8.4	Reagent and QC Loading Instructions
1	When Reagent/QC material is reconstituted and ready for use proceed to step 2
2	From Home Page select **Loading – Products**
3	Scan the Reagent/QC
4	The Instrument will ask whether the volume is correct, or it needs to be modified.
5	Accept or modify the volume then press **Enter**
6	Load the Reagent/QC which was just scanned. **Note:** Neoplastine CI Plus reagent vial requires to be sitting in the position which is systematically stirred by a lateral movement.

NOTE: In the event that the test system becomes inoperable, notify supervision or designee for further direction. Patient specimens must be stored in a manner that maintains the integrity of the specimen.

9. CALCULATIONS

The INR is calculated by the STA Compact and transmitted to the LIS computer.

$$\text{INR} = \left(\frac{Patient's\ PT}{Geometric\ Mean\ PT} \right)^{\text{ISI}}$$

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The INR is automatically calculated by the STA® Compact. The ISI is furnished by the manufacturer in the package insert and is stored in the CALIBRATION page for PT (or PT+) along with the geometric mean (reference time).

10. REPORTING RESULTS AND REPEAT CRITERIA

10.1 Interpretation of Data

N/A

10.2 Rounding

Results are reported out in seconds to the nearest 0.1 sec.

10.3 Units of Measure

Seconds

10.4 Clinically Reportable Range (CRR)

10 - 120 seconds

10.5 Review Patient Data

Each result is reviewed for error messages. Refer to the STA® Compact system manual “Error messages” section for troubleshooting. Resolve any problems noted before issuing patient reports.

10.6 Repeat Criteria and Resulting

The printout from the STA Compact is reviewed for repeat criteria and samples are repeated if needed. Results will be transmitted to the LIS and released using the OEM function.

IF the result is …	THEN…
INR > 3.9 seconds	Check for clots, but there is no need to repeat. Be sure the specimen is not under-filled or over-filled, then check the Hematocrit (HCT) result. If the HCT is greater than 55%, refer to appendices A and B for special tube preparation.
>Mmax	Repeat, check for clots. If result is still > Mmax, report with >120 and INR is reported as >16.2

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< Mmin	Repeat, check for clots, most likely this sample clotted during collection. If no clots are detected and the repeat matches, Report as < 10. Report the INR < 0.7.

11. EXPECTED VALUES

11.1 Reference Ranges

PT 12.5 – 14.8 seconds

11.2 Critical Values

INR > 3.9

11.3 Standard Required Messages

The following INR comment is automatically added to the report by the LIS:

The American College of Chest Physicians/National Heart/Lung Institute has recommended MODERATE INTENSITY ANTICOAGULATION REGIMEN INR 2.0-3.0 for most indications with the exception of patients with MECHANICAL PROSTHETIC HEART VALVES AND RECURRENT EMBOLISM for whom the recommended range is INR 2.0-3.5.

12. CLINICAL SIGNIFICANCE

The Prothrombin Time (PT) is a basic coagulation-screening test that is useful in the assessment of congenital and acquired extrinsic coagulation pathway deficiencies involving factors II, V, VII and X. The International Normalized Ratio (INR) is a means of standardizing therapeutic range interpretation of the PT. The use of the INR is limited to the assessment of PT in patients on oral anticoagulant therapy.

If a PT monitored factor deficiency is present, immediate and incubated mixing studies will correct to normal. If an inhibitor is present, the immediate result **may** correct, but the incubated result will be abnormal.

13. PROCEDURE NOTES

- **FDA Status:** Approved/cleared
- **Validated Test Modifications:** None

The STA uses electromechanical clot detection for the Protime. Lipemia and icterus do not interfere with PT result. These findings should be reported with the PT value. The STA® is programmed to detect the prothrombin times from 10 seconds to 120 seconds. Prothrombin

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Times that clot in less than 10 seconds will yield a <Vmin result and Prothrombin Times that do not clot in 120 seconds yield a >Vmax result.

New lot of Thromboplastin: With each new lot of thromboplastin, a patient geometric mean time must be established. The operator must enter that geometric mean time before the STA®/STA® Compact will allow QC to be run. Through the MAIN MENU select CALIB/CONTROL. Select **CALIBRATION**, press **Enter** ↵. Cursor to the PT (or PT+) test and press **Enter** ↵ to select. Press ESC key for options. Select MODIFY REFERENCE TIME/RANGE, press **Enter** ↵Type in the Geometric Mean Time and save with **F10**. This screen also stores the ISI value, as downloaded from the reagent bar code sheet.

INR Verification: The INR calculation will be manually verified with each new reagent lot number and with the semi-annual instrument-to-instrument comparison.

14. LIMITATIONS OF METHOD

14.1 Analytical Measurement Range (AMR)

10 – 120 seconds

14.2 Precision

Different plasmas were used for the intra assay and inter assay reproducibility studies on the STA® Compact.

	Intra-Assay Reproducibility		Inter-Assay Reproducibility	
Sample	**Sample 1**	**Sample 2**	**Sample 3**	**Sample 4**
n	21	21	10	10
mean (seconds)	13.6	22.7	15.1	29.4
SD (seconds)	0.10	0.12	0.22	0.46
CV (%)	0.7	0.5	1.5	1.6

14.3 Interfering Substances

PT results will not be affected by levels of unfractionated heparins up to 1 IU/mL and by LMWH up to 1.5 anti-Xa IU/mL.
Many commonly administered drugs affect the results obtained in PT testing. (Example: coumadin and heparin).
STA® Neoplastine CI Plus, contain a specific inhibitor of heparin. Therefore, only levels of heparin outside of the therapeutic range will affect the PT results.

14.4 Clinical Sensitivity/Specificity/Predictive Values

N/A.

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15. SAFETY

Refer to your local and corporate safety manuals and Safety Data Sheet (SDS) for detailed information on safety practices and procedures and a complete description of hazards.

16. RELATED DOCUMENTS

1. Laboratory Quality Control Program
2. Laboratory Safety Manual
3. Safety Data Sheets (SDS)
4. Hemolysis, Icteria and Lipemia Interference (Lab policy)
5. Repeat Testing Requirements (Lab policy)
6. Critical Values (Lab policy)
7. STA Compact Operating Instructions, Coagulation procedure
8. Verification of Platelet Poor Plasma, Coagulation procedure
9. Current package insert for STA® Neoplastine CL Plus Prothrombin Time

17. REFERENCES

1. Diagnostic Stago Neoplastine CL Plus package insert: Revised 6/2015.
2. STA®-Coag Control N + ABN Plus (REF 00677): citrated control plasmas normal and abnormal levels; Control Plasmas for Assays of Coagulation Parameters on STA®, Revised 09/2014.
3. STA® Compact Operators Manual. STA® DSI-TSD-SM August 2004, STA® DSI-TSD-US April 2003, and V1.3 revised February 2003.
4. Quest Diagnostics Nichols Institute in Chantilly, VA. SOP ID QDHE716 Version 3.1, Coagulation Specimen Collection and Handling in 3.2% Sodium Citrate Blue Topped Tubes.
5. Reagents for STA® Compact Line, Reconstitution and Handling Information, revised 02/20/2009.

18. REVISION HISTORY

Version	Date	Section	Reason	Reviser	Approval
			Supersedes G004.005		
000	06/08/12	4.1	Remove Millipore water	J. Buss	J. Buss, RSL
000	06/08/12	6.3	Add QC performed after maintenance	J. Buss	J. Buss, RSL
000	06/08/12	15	Update to standard wording	L. Barrett	J. Buss, RSL
001	6/3/14		Update owner	L Barrett	R SanLuis
001	6/3/14	1	Update order code	A Chini	R SanLuis
001	6/3/14	3.1	Add reference to Appendices	A Chini	R SanLuis
001	6/3/14	3.2	Update tube volumes, add opened container storage	A Chini	R SanLuis

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001	6/3/14	11.3	Remove calling PAT and SDS	L Barrett	R SanLuis
001	6/3/14	10.5	Add reference to Appendices	A Chini	R SanLuis
001	6/3/14	16	Update titles	L Barrett	R SanLuis
001	6/3/14	19	Add Appendix A and B	A Chini	R SanLuis
001	6/3/14	Footer	Version # leading zero's dropped due to new EDCS in use as of 10/7/13.	L Barrett	R SanLuis
2	4/2/15	6.4, 6.6	Replace LIS with Unity Real Time	L Barrett	R SanLuis
3	4/22/16	3.2	Change whole blood to Plt poor plasma, update stability, add over-filled tubes as unacceptable	A Chini	R SanLuis
3	4/22/16	4.2, 8.3	Add stir bar decontamination	A Chini	R SanLuis
3	4/22/16	5	Add explanation for STA QC and Bio-Rad QC. Add STA QC info.	A Chini	R SanLuis
3	4/22/16	6.1, 6.2	Update to Bio-Rad QC	A Chini	R SanLuis
3	4/22/16	6.2	Add instruction for loading onboard	A Chini	R SanLuis
3	4/22/16	6.5	Update review patient data criteria	A Chini	R SanLuis
3	4/22/16	6.7	Add TEa criteria and QC submitted to Bio-Rad on monthly	A Chini	R SanLuis
3	4/22/16	8.1	Specify temperature checks every shift	A Chini	R SanLuis
3	4/22/16	11.2	Reformat value to eliminate ≥ sign	L Barrett	R SanLuis
3	4/22/16	17	Add Bio-Rad QC	A Chini	R SanLuis
4	7/25/16	5.1	Removed Bio-Rad QC information	A Chini	R SanLuis
4	7/25/16	6.1, 6.2	Replace Bio-Rad QC with STA Coag Controls	A Chini	R SanLuis
4	7/25/16	6.7	Remove QC submission to Bio-Rad	A Chini	R SanLuis
4	7/25/16	8.4	Add QC/Reagent Loading Instructions	A Chini	R SanLuis
4	7/25/16	11.3	Move report comment from 10.5	L Barrett	R SanLuis
4	7/25/16	17	Remove Bio-Rad insert	A Chini	R SanLuis
5	5/1/17	Header	Add other sites	L Barrett	R SanLuis
5	5/1/17	4, 6	Remove individual section labeling instructions and add general one	L Barrett	R SanLuis
5	5/1/17	6.3	Remove run QC at beginning of shift	L Barrett	R SanLuis
5	5/1/17	8.3	Specify 5 minute soak with stirring	L Barrett	R SanLuis
5	5/1/17	10.5	Review data moved from section 6	L Barrett	R SanLuis
5	5/1/17	15	Update to new standard wording	L Barrett	R SanLuis

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19. ADDENDA

A. Instructions for Preparing Collection Tube for Hematocrit >55%
B. Phlebotomist Instructions for Blood Collection

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Appendix A

Instructions for Preparing Collection Tube for Hematocrit >55%

Explanation:

Polycythemia is a disease state in which the proportion of blood volume that is occupied by red blood cells increases - basically when Hematocrit (HCT) is greater than 55%. It can cause prolonged coagulation results.
When a prolonged coagulation result is obtained, check the specimen for a clot first.
If the specimen is not clotted, be sure the specimen is not under-filled or over-filled, then check the HCT result.
If a HCT result of greater than 55% is obtained, immediately notify the doctor or attending nurse and ask for a redraw using a special tube prepared by the lab.

To prepare a special tube in the lab use the following instructions and formula:

The anticoagulant volume in the collection tube must be adjusted to obtain a 9:1 ratio of blood to Sodium Citrate. Under or over-filling of the specially prepared collection tube is not acceptable.
The vacuum in the collection tube will be broken to adjust the volume of collection anticoagulant. Because of this special collection technique, the stability for these whole blood specimens is reduced to four (4) hours after collection.

Formula to calculate the anticoagulant volume is:
Anticoagulant in mL = [(100 – HCT) / (595 – HCT)] x Volume of blood

Example 1: Specimen with a 70% HCT in a 2.7 mL tube:

Patient with HCT of 70%
Using a 2.7 mL tube
Anticoagulant in mL = [(100 – 70) / (595 – 70)] x 2.7 = 0.15 mL or 150 uL
Pipette a 2.7 mL tube in a way to leave only 150 uL of anticoagulant in there.
A 2.7 mL tube contains 0.3mL anticoagulant; therefore remove 0.15mL

Example 2: Specimen with a 70% HCT in a 1.8 mL tube:

Patient with HCT of 70%
Using a 1.8 mL tube
Anticoagulant in mL = [(100 – 70) / (595 – 70)] x 1.8 = 0.1 mL or 100 uL
Pipette a 1.8 mL tube in a way to leave only 100 uL of anticoagulant in there.
A 1.8 mL tube contains 0.2mL anticoagulant; therefore remove 0.1mL

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Example 3: Specimen with a 60% HCT in a 2.7 mL tube:

Patient with HCT of 60%
Using a 2.7 mL tube
Anticoagulant in mL = [(100 – 60) / (595 – 60)] x 2.7 = 0.2 mL or 200 uL
Pipette a 2.7 mL tube in a way to leave only 200 uL of anticoagulant in there.
A 2.7 mL tube contains 0.3mL anticoagulant; therefore remove 0.1mL

Example 4: Specimen with a 60% HCT in a 1.8 mL tube:

Patient with HCT of 60%
Using a 1.8 mL tube
Anticoagulant in mL = [(100 – 60) / (595 – 60)] x 1.8 = 0.13 mL or 130 uL
Pipette a 1.8 mL tube in a way to leave only 130 uL of anticoagulant in there.
A 1.8 mL tube contains 0.2mL anticoagulant; therefore remove 0.07mL

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Appendix B

Phlebotomist Instructions for Blood Collection

The technologist will prepare a special tube in which the anticoagulant has been adjusted, therefore the tube is not vacuumed. The technologist will inform the phlebotomist of the exact amount of blood needed to fill the tube.

Equipment and Supplies

Latex free gloves
Latex free tourniquet
Latex free Band Aid or Tape
Alcohol Prep (70% alcohol)
2x2 sterile gauze
Collection tube
Blood Collection Set 21 or 23 gauge winged set
Blood Transfer Device
3mL syringe
Biohazard bag
Biohazard sharps container
LIS collection list and label/Lab requisition

Collection Steps

1. Introduce yourself to the patient by stating your first and last name.
2. Positively identify the patient according to the SOP 'Patient Identification', Phlebotomy procedure manual.
3. Wash hands. Apply gloves.
4. Explain the procedure to the patient and obtain patient's consent to draw blood.
5. Collect equipment and correct technologist-provided collection tube.
6. Assemble equipment and break needle and syringe seals in the presence of the patient.
7. Apply tourniquet about midway between the elbow and the shoulder 3-4 inches above the venipuncture site). Place patient's arm in a downward position to prevent reflux of 'backflow' of blood from the tube into the venous system. Ask the patient to close hand gently.
8. Palpate/feel for vein locating a vein that will flow fast (reducing the possibility of the blood clotting).
9. Clean the area for venipuncture with a 70% alcohol pad decontaminating the collection site.
10. Allow the area to air-dry completely.
11. Assemble the 21 or 23 gauge winged set to the 3mL syringe. Pull back the plunger to dispel all the air out of the syringe.
12. With the bevel up, align the needle with the vein while holding the skin taut. Insert the needle at a 15-30 degree angle with the skin. Remove your hand from drawing the skin taut. Grasp the syringe and draw back bringing the plunger tip to the exact amount of blood requested by the technologist.
13. Release the tourniquet. Ask the patient to open hand.

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14. Place gauze above the puncture site and remove the needle while simultaneously applying pressure on the puncture sit. Firmly activate needle safety shield, a click must be heard to ensure that the safety shield is secure.
15. Remove 21 or 23 gauge winged set from syringe.
16. Attach the blood-filled syringe to the Blood Transfer Device.
17. Connect the Blood Transfer Device to the un-vacuumed tube, provided by the technologist, and slow and gently fill the collection tube. DO NOT FORCE blood into tube. Pressure can lead to tube explosion and blood exposure.
18. Place the cap on the tube and invert a few times to make sure the anticoagulant is mixed with blood.
19. Dispose of all blood collection equipment into the nearest sharps container. DO NOT disassemble the syringe from the Blood Transfer Device.
20. Dispose of all other used materials in appropriate container and wash hands.
21. Label the sample with the LIS collection label and write the time, date, and your tech code. Transport specimen to the Lab.

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Technical SOP

Title	**Fibrinogen**	
Prepared by	Ashkan Chini	Date: 4/7/2011
Owner	Robert SanLuis	Date: 4/7/2011

Laboratory Approval	**Local Effective Date:**	
Print Name and Title	**Signature**	**Date**
Refer to the electronic signature page for approval and approval dates.		

Review		
Print Name	**Signature**	**Date**

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Quest Diagnostics
Site: Shady Grove Medical Center, Washington Adventist Hospital
Title: **Fibrinogen**

TABLE OF CONTENTS

1. TEST INFORMATION

Assay	Method/Instrument	Local Code
Fibrinogen, Quantitative	Clot based assay / STA® Compact	FIBR

Synonyms/Abbreviations
FIB

Department
Coagulation

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2. ANALYTICAL PRINCIPLE

The STA® Fibrinogen kit is intended for the quantitative determination of fibrinogen in plasma by the clotting method of Clauss. In the presence of an excess of thrombin, the clotting time of diluted plasma is inversely proportional to the level of plasma fibrinogen. The clot is detected by the STA® Compact. The STA® Compact is a fully automated coagulation instrument that uses an electromagnetic mechanical clot detection system. The oscillation of a steel ball within the cuvette with the thrombin and diluted plasma is monitored by the STA® Compact. When the oscillation of the steel ball is stopped by clot formation, the sensor registers the time in seconds. The time is read from a stored curve on the STA® Compact. An increase of the fibrinogen level is observed in cases of diabetes, inflammatory syndromes and obesity. A decrease of the fibrinogen level is observed in DIC, fibrinolysis, thrombolytic therapy and hereditary diseases.

3. SPECIMEN REQUIREMENTS

3.1 Patient Preparation

Component	Special Notations
Fasting/Special Diets	N/A
Specimen Collection and/or Timing	Normal procedures for collecting plasma may be used for samples to be analyzed by this method. Vacutainer tube must be filled to the line to ensure the proper ratio of blood to anticoagulant.
Special Collection Procedures	If hematocrit >55%, refer to appendices A and B for collection instructions.
Other	When the fibrinogen assay is to be performed on samples collected from patients receiving thrombolytic therapy, the blood samples must be collected with an anti-coagulant mixture containing a plasmin inhibitor (See section 13).

3.2 Specimen Type & Handling

Criteria	
Type -Preferred **-Other Acceptable**	PLT Poor Plasma (sodium citrate) None
Collection Container	Light blue top tube (3.2% sodium citrate) Citrated blood 9:1 (blood to anticoagulant)
Volume - Optimum **- Minimum** **- Optimum** **- Minimum**	2.7 mL (9:1 blood to anticoagulant) in a 2.7 ml tube 2.4 mL (9:1 blood to anticoagulant) in a 2.7 ml tube 1.8 mL (9:1 blood to anticoagulant) in a 1.8 mL tube 1.8 mL (9:1 blood to anticoagulant) in a 1.8 mL tube
Transport Container and Temperature	Light blue vacutainer (as above) or a clean plastic screw capped vial at room temperature.

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Criteria		
Stability & Storage Requirements	Room Temperature:	4 hours
	Refrigerated:	4 hours (PLT Poor Plasma)
	Frozen plasma: -20C or colder	2 weeks (PLT Poor Plasma)
Specimen preparation	Centrifuge whole blood for specified time /speed documented on each centrifuge for preparing platelet-poor plasma.	
Unacceptable Specimens & Actions to Take	Specimens that are unlabeled, improperly labeled, or those that do not meet the stated criteria are unacceptable. Clotted, under-filled or over-filled tubes are not accepted. Request a recollection and credit the test with the appropriate LIS English text code for "test not performed" message.	
Compromising Physical Characteristics	Moderate to gross hemolysis. Reject sample and request a recollection. Credit the test with appropriate LIS English text code HMM (Specimen moderately hemolyzed) or HMT (Specimen markedly hemolyzed) Lipemia: Acceptable Icterus: Acceptable	
Other Considerations	None	

NOTE: Labeling requirements for all reagents, calibrators and controls include: (1) Open date, (2) Substance name, (3) Lot number, (4) Date of preparation, (5) Expiration date, (6) Initials of tech, and (7) Any special storage instructions. Check all for visible signs of degradation.

4. REAGENTS

The package insert for a new lot of kits must be reviewed for any changes before the kit is used. A current Package Insert is included as a Related Document.

4.1 Reagent Summary

Reagents	Supplier & Catalog Number
STA – Fibrinogen	Diagnostic Stago (REF 00674)
STA – Owren-Koller Buffer	Diagnostic Stago (REF 00360)
Pure Reagent Grade water	Millipore or NERL Thermo Scientific (Cat. No. 0015)

4.2 Reagent Preparations and Storage

Reagent 1	**STA – Fibrinogen**
Container	Manufacturer supplied vial
Storage	2-8°C

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Stability	Stable until expiration date indicated on the box label. Once reconstituted, the reagent is stable: • 5 days with perforated plastic cap in place. • 14 days at 2-8°C in its original capped vial.
Preparation	Reconstitute each vial with 5 mL of reagent grade water. Allow the reconstituted reagent to stand at room temperature (18-25°C) for 30 minutes. Swirl vial gently. Then place the perforated plastic cap on the vial.

Reagent 2	**STA – Owren-Koller Buffer**
Container	Manufacturer supplied vial
Storage	2-8°C
Stability	The buffer solution in intact bottles is stable until the expiration date indicated on the box label. After opening it remains stable for 3 days.
Preparation	Allow it to stand at room temperature (18-25°C) for 30 minutes before use.

Reagent 3	**NERL Reagent Grade water**
Container	Manufacturer supplied vial
Storage	Room temperature
Stability	Stable 30 days after opening.
Preparation	Ready to use

5. CALIBRATORS/STANDARDS

5.1 Calibration Procedure

The pre-calibrated Fibrinogen values are identical for all the vials of each lot.
To enter the calibration data on the analyzer, scan the barcode printed on the assay value insert across the instrument barcode reader.
The calibration data will be validated for the lot being used once the Stago Fibrinogen controls are run and tested.

The calibration curve is considered verified for the new reagent lot when both the STA®-Coag Control N + ABN Plus are within acceptable range. The acceptable STA®-Coag Control N + ABN Plus range is supplied by Stago. QC ranges must fall within the acceptable range which is established utilizing the peer group data in combination with our current/historic analytic performance.

6. QUALITY CONTROL

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6.1 Controls Used

Controls	Supplier and Catalogue Number
STA - Coag Control N + ABN Plus	Stago Diagnostics, Cat. No. 00677

6.2 Control Preparations and Storage

Control	**STA - Coag Control N + ABN Plus**
Preparation	Reconstitute each vial of Reagent 1 or 2 with exactly 2 mL of reagent grade water. Allow the reconstituted material to stand at room temperature (18 - 25°C) for 30 minutes. Then, mix by turning the vial upside down, 3 – 4 times, to obtain a homogeneous solution.
Storage	2 - 8°C
Stability	The reagents in intact vials are stable until the expiration date indicated on the box label, when stored at 2 - 8°C. Once reconstituted, Reagents 1 and 2 remain stable in their original vials for 24 hours on STA Compact.

1. QC can be run automatically at pre-set intervals (in Test Set-up) or by ordering manually from the Quality Control Menu.
2. All control ranges are monitored automatically by the STA® Compact. If any controls are outside the ± 2 SD range, the instrument will audibly and visually alarm the operator. Otherwise, the results can be found in the individual QC files. Control results are automatically filed in the STA® Compact QC file. All results for a 24-hour period are converted to a "mean" value at midnight. This mean is used in the statistical data and is plotted on the Levy-Jennings chart as a daily mean.
3. To print all the QC data points for the Fibrinogen test, perform the following procedure prior to midnight. From the MAIN MENU under CAL. /CONTROL select QUALITY CONTROL press **Enter** ↵. Cursor to the FIB test and press **Enter** ↵to view the Levy-Jennings chart. Press **F1** to view the results in tabular form. Press **F6, select Execute then** press **Enter** ↵to print the individual values under current controls. Press ESC key to exit (back to graph). Press **F2** or **F3** to view other levels and continue with **F1** to view the result list.

6.3 Frequency

Controls are run ~~at the beginning of each shift and~~ every 4 hours of patient testing and with the change of any reagent used in test performance.

Controls are run after any maintenance is performed on the analyzer.

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6.4 Tolerance Limits and Criteria for Acceptable QC

Step	Action
1	The established QC ranges are in the QC file of the STA Compact. The quality control results from the instrument are transmitted to Unity Real Time and can be viewed in that program. Any out-of-range QC results will be flagged.
2	If all controls are within QC parameters all sample results can be reported.
3	Rejected runs must be effectively addressed by corrective action. Steps taken in response to QC failures must be documented. Patient samples in failed analytical runs must be reanalyzed. Supervisor may override rejection of partial or complete runs only with detailed documentation that follows criteria that is approved by the Medical Director.
4	Corrective action documentation must include the following: QC rule(s) violated, the root cause of the problem, steps taken to correct the problem, how patient samples were handled, and the date and initials of the person recording the information. See the QA SOP "QC Responsibilities and Review" for more detail.
5	If the assay is down and results will not be reported in the scheduled turnaround time, clients will be notified of the situation.

6.5 Documentation

- QC tolerance limits are programmed into the instrument and Unity Real Time; it calculates cumulative mean, SD and CV and stores all information for easy retrieval.
- Quality control records are reviewed daily at the bench, weekly by the Group Lead or designee, and monthly by the Supervisor/Manager or designee.
- Refer to complete policies and procedures for QC documentation and for record retention requirements in the Laboratory QC Program.

6.6 Quality Assurance Program

- Each new lot number of reagent or new shipment of the same lot of reagent must be tested with external control materials and previously analyzed samples. Performance of the new lot must be equivalent to the previous lot; utilize published TEA for acceptability criteria.
- Training must be successfully completed and documented prior to performing this test. This procedure must be incorporated into the departmental competency assessment program.
- The laboratory participates in CAP proficiency testing. All proficiency testing materials must be treated in the same manner as patient samples.
- Monthly QC must be presented to the Medical Director or designee for review and signature.
- Consult the Laboratory QC Program for complete details.

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7. EQUIPMENT and SUPPLIES

7.1 Assay Platform

STA® Compact – Analyzer

7.2 Equipment

- Refrigerator capable of sustaining 2–8°C.
- Freezer capable of sustaining range not to exceed -20 to -70°C.
- Centrifuge calibrated for preparing platelet-poor plasma

7.3 Supplies

- Cuvette Roll – Diagnostic Stago
- STA – black adaptors
- STA – brass adaptors
- Plastic micro cups
- Plastic transfer pipettes
- Micro sample tube – Diagnostic Stago

8. PROCEDURE

NOTE: For all procedures involving specimens, buttoned lab coats, gloves, and face protection are required minimum personal protective equipment. Report all accidents to your supervisor.

8.1	Instrument Set-up Protocol
1	At the start of each shift, verify instrument temperatures and availability of cuvettes and cleaner solution by accessing the System Status screen from the main bar.
2	Verify instrument temperatures every shift and record on the maintenance sheet. If the Needle number 3, measuring block, or reagent drawer temperatures are out of range, corrective action must be taken prior to patient samples being run.
3	Make sure that there is an adequate supply of reagents in the analyzer, and they are in date.
4	Load cuvettes and cleaner/wash solution on the analyzer if needed.

8.2	Analytical Procedure
1	Refer to START-UP procedure for STA® Compact before running patient specimens on the STA® Compact at the start of each shift.
2	Request quality control. Through MAIN MENU under CALIB. /CONTROL select QUALITY CONTROL and press **Enter** ↵. Cursor to the FIB test. Select FIB by pressing **F1** and then **F10.** Type in your Access Code to run the QC.

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8.2	Analytical Procedure
3	Load patients' samples: Access the sample drawer(s) through the MAIN MENU, under LOADING, Select Sample, press **Enter** ↵. After the drawer opens, identify the type of specimen, such as micro sample (press **F8**), or stat (press **F12**). Identify the sample by bar coding or manually entering on the keyboard the patient identification number and then placing the specimen into the drawer.
4	In MANUAL MODE, the operator must order the test(s) from the Selection menu or from the Recorded Profile/s Cursor to the test and press **Enter** ↵to select. When all tests are ordered, press F10 to save.
5	In AUTO MODE, the STA®/STA® Compact will automatically order the test(s) selected in the AUTO MODE profile.
6	If TELELOADING is selected as the AUTO MODE profile, the STA®/STA® Compact will query the host computer and download the test(s) as well as assign the status (i.e. stat).
7	As soon as the sample drawer closes, the TEST STATUS screen will appear. If there is not enough reagent(s) to run the test(s), the suspect reagent(s) will appear in red with the amount of depletion. This depletion of reagent will BLOCK the SAMPLE PIPETTING. When this occurs, add the necessary reagent(s) to run the samples by responding N (NO) to the warning message 'NEW TESTS ARE DELAYED - REACTIVATE?' Reagents can then be loaded in the drawer. By responding Y (YES) to the warning message 'NEW TESTS ARE DELAYED - REACTIVATE?', the instrument will continue to perform all tests for which there is sufficient reagent (i.e. while waiting for reagents to stabilize after reconstitution)
8	All patient results are displayed on the TEST PANEL screen and automatically print out and transmit if selected on the system status menu.
9	For results in question that need operator intervention, cursor to the identification number in the TEST PANEL screen and press enter. This will display the FILE PROCESSING screen. Follow the options on the left-hand side of the screen (i.e. **F3** - rerun test).

8.3	Reagent and QC Loading Instructions
1	When Reagent/QC material is reconstituted and ready for use proceed to step 2
2	From Home Page select **Loading** – **Products**
3	Scan the Reagent/QC
4	The Instrument will ask whether the volume is correct, or it needs to be modified.
5	Accept or modify the volume then press **Enter**
6	Load the Reagent/QC which was just scanned. **Note:** Neoplastine CI Plus reagent vial requires to be sitting in the position which is systematically stirred by a lateral movement.

NOTE: In the event that the test system becomes inoperable, notify supervision or designee for further direction. Patient specimens must be stored in a manner that maintains the integrity of the specimen.

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9. CALCULATIONS

The STA® Compact automatically converts the results in seconds from a standard curve (log-log) to mg/dL. The assay uses a dilution of 1:20 sample plasma to buffer. The STA® System automatically dilutes this sample to a 1:8 dilution on samples with a concentration <150 mg/dL or a 1:40 dilution if the value is >900 mg/dL. If the auto redilute feature is necessary the results are displayed on the Screen in Blue numerals, instead of the normal Black numerals.

10. REPORTING RESULTS AND REPEAT CRITERIA

10.1 Interpretation of Data

N/A

10.2 Rounding

No rounding is necessary. The instrument reports results as a whole number.

10.3 Units of Measure

mg/dL

10.4 Clinically Reportable Range (CRR)

60 - 1800 mg/dL

10.5 Review Patient Data

Each result is reviewed for error messages. Refer to the STA® Compact system manual "Error messages" section for troubleshooting. Resolve any problems noted before issuing patient reports.

10.6 Repeat Criteria and Resulting

The printout from the STA Compact is reviewed for repeat criteria and samples are repeated if needed. Results will be transmitted to the LIS and released using the OEM function.

IF the result is …	THEN…
< Mmin	Repeat, check for clots before reporting results as: >1800 mg/dL, REP
>Mmax	Repeat, check for clots before reporting results as: <60 mg/dL, REP
< 100	Repeat, report with comment "REP"

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IF the result is …	THEN…
> 800	Repeat, report with comment "REP"
For any of the above situations, be sure the specimen is not under-filled or over-filled, and then check the Hematocrit (HCT) result. If the HCT is greater than 55%, refer to appendices A and B for special tube preparation.	

Definitions:

<Mmin: The shortest time limit below which no result will be given. In the case of Fibrinogen this means the value is greater than 1800 mg/dL

>Mmax: The longest time limit above which no result will be given. In the case of Fibrinogen this means the value is less than than 60 mg/dL

Special notes related to fibrinogen results:

- A >Mmax for the result for Fibrinogen means the Fibrinogen value is **extremely low**.
- A <Mmin result for Fibrinogen means the Fibrinogen value is **extremely high**.
- See Note # 1 in section 13. It is possible to have a >Mmax or <Mmin result after the instrument does the auto redilutes.

11. EXPECTED VALUES

11.1 Reference Ranges

200 – 400 mg/dL

11.2 Critical Values

< 100 mg/dL
> 800 mg/dL

11.3 Standard Required Messages

None established

12. CLINICAL SIGNIFICANCE

An increase of fibrinogen level is found in cases of diabetics, inflammatory syndromes, obesity, and pregnancy. A decrease of the fibrinogen is observed in DIC and fibrinogenolysis. Furthermore, fibrinogen seems to be involved in the pathogenicity of the thrombotic cardiovascular events. Fibrinogen is composed of six chains: two alpha, two beta and two gamma chains. Thrombin (factor IIa) breaks up the fibrinogen molecule to split out two fibrinopeptide fragments from the A*α* chain and two fibrinopeptide fragments from the Bβ chain. The fibrin monomers that are produced from these reactions then aggregate to form fibrin, which is subsequently stabilized by factor XIIIa. The first step of this stabilization consists of the binding of

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two *γ* chains of two fibrin monomers. This binding is the origin of the D-Dimer, the degradation product that is specific of fibrin.

13. PROCEDURE NOTES

- **FDA Status:** Approved/cleared
- **Validated Test Modifications:** None

1. The STA uses electro-mechanical clot detection test, therefore lipemia and icterus do not interfere with the fibrinogen result. These findings should be reported with the results.
2. When the STA® Compact redilutes a patient sample at a more appropriate dilution (as pre-determined in Test Set-up) the results in the TEST PANEL screen which appear in Blue numerals have already been corrected by the STA® Compact for the dilutional difference.
3. Patients receiving thrombolytic therapy will have a rapid drop in the plasma Fibrinogen level and these samples **MUST** be collected with an anticoagulant containing a plasmin inhibitor such as Aprotinin, Cat. No. 0820, to determine an accurate Fibrinogen result.

14. LIMITATIONS OF METHOD

14.1 Analytical Measurement Range (AMR)

150 – 900 mg/dL

14.2 Precision

Different plasmas were used for reproducibility studies with the STA Fibrinogen Results obtained on the STA analyzer are shown in the package insert.

14.3 Linearity

The package insert states that the working range of the reagent on the STACompact® System instrument is 150-900 mg/dL. This is at the normal dilution (1:20) which, the instrument uses to assay samples. The linearity range on the STA® System instrument is 60-1800 mg/dL (see the bar-coded Calibration Curve) due to the different dilutions used for the auto redilution: 1:8 if < 150 mg/dl and 1:40 if > 900 mg/dL. For extremely high Fibrinogen samples a higher dilution can be set up as a dependent test.

14.4 Interfering Substances

1. In patients receiving drugs that affect the fibrinolytic system, the plasma levels of fibrinogen degradation products (FDP) may be extremely high. FDPs may inhibit both thrombin action of fibrinogen and fibrin polymerization. At normal fibrinogen concentrations, FDPs have a minimal effect on the fibrinogen assay.

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At fibrinogen concentrations below 150 mg/dL, FDPs greater than 130 µg/mL increasingly inhibit the thrombin clotting rate assay. High levels of paraproteins may interfere with the polymerization of fibrin monomers.

2. The clinical use of topical bovine thrombin has led to the generation of antibodies in some patients. These antibodies may lead to artifactual prolongation of the thrombin clotting rate assay of fibrinogen.
3. Heparin may interfere with this assay. However, the STA®-Fibrinogen reagent contains a specific inhibitor of heparin. Any prolongation of the assay is therefore, related to a real coagulation factor deficiency of Fibrinogen.

15. SAFETY

Refer to your local and corporate safety manuals and Safety Data Sheet (SDS) for detailed information on safety practices and procedures and a complete description of hazards.

16. RELATED DOCUMENTS

1. Laboratory Quality Control Program
2. Laboratory Safety Manual
3. Safety Data Sheets (SDS)
4. Hemolysis, Icteria and Lipemia Interference (Lab policy)
5. Repeat Testing Requirements (Lab policy)
6. Critical Values (Lab policy)
7. STA Compact Operating Instructions, Coagulation procedure
8. Verification of Platelet Poor Plasma, Coagulation procedure
9. Current package insert for STA® Fibrinogen

17. REFERENCES

1. Diagnostic Stago Fibrinogen package insert: Revised 01/2016.
2. STA®-Coag Control N + ABN Plus (REF 00677): citrated control plasmas normal and abnormal levels; Control Plasmas for Assays of Coagulation Parameters on STA®, Revised 09/2014.
3. STA® Compact Operators Manual. STA® DSI-TSD-SM August 2004, STA® DSI-TSD-US April 2003, and V1.3 revised February 2003.
4. Diagnostic Stago Owren – Koller buffer solution for coagulation tests, revised 05/2014.
5. Clauss A,"Rapid Physiological Coagulation Method for the Determination of Fibrinogen [German], *"Acta Haematol,* 1957,17:237-46.
6. Quest Diagnostics Nichols Institute in Chantilly, VA. SOP ID QDHE716 Version 3.1, Coagulation Specimen Collection and Handling in 3.2% Sodium Citrate Blue Topped Tubes.

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18. REVISION HISTORY

Version	Date	Section	Reason	Reviser	Approval
			Supersedes G002.005		
000	10/31/11	10.5	Revise MMin to > 1800 mg/dl and MMax to <60 mg/dl; add special notes	C Reidenauer	C Reidenauer
000	10/31/11	15	Update to standard wording	L Barrett	C Reidenauer
000	10/31/11	17	Add reference 5	C Reidenauer	C Reidenauer
001	10/19/12	3.2	Delete frozen storage	C Reidenauer	R SanLuis
001	10/19/12	4.1	Remove Millipore water	L Barrett	R SanLuis
002	6/3/14	3.1	Add reference to Appendices	A Chini	R SanLuis
002	6/3/14	3.2	Update tube volumes, add opened container storage	A Chini	R SanLuis
002	6/3/14	4.2	Change storage temp and prep for buffer	A Chini	R SanLuis
002	6/3/14	6.2	Add step to print QC	A Chini	R SanLuis
002	6/3/14	10.5	Add instruction for Hct >55	A Chini	R SanLuis
002	6/3/14	11.1	Change upper limit form 500 to 400	A Chini	R SanLuis
002	6/3/14	16	Update titles	L Barrett	R SanLuis
002	6/3/14	17	Add references 6	A Chini	R SanLuis
002	6/3/14	19	Add Appendix A and B	A Chini	R SanLuis
002	6/3/14	Footer	Version # leading zero's dropped due to new EDCS in use as of 10/7/13.	L Barrett	R SanLuis
3	4/2/15	6.4, 6.6	Replace LIS with Unity Real Time	L Barrett	R SanLuis
4	4/22/16	3.2	Change whole blood to Plt poor plasma, update stability, add over-filled tubes as unacceptable	A Chini	R SanLuis
4	4/22/16	5	Add explanation for STA QC and Bio-Rad QC. Add STA QC info.	A Chini	R SanLuis
4	4/22/16	6.1, 6.2	Update to Bio-Rad QC	A Chini	R SanLuis
4	4/22/16	6.2	Add instruction for loading onboard	A Chini	R SanLuis
4	4/22/16	6.5	Update review patient data criteria	A Chini	R SanLuis
4	4/22/16	6.7	Add TEa criteria and QC submitted to Bio-Rad on monthly	A Chini	R SanLuis
4	4/22/16	8.1	Specify temperature checks every shift	A Chini	R SanLuis
4	4/22/16	17	Add Bio-Rad QC	A Chini	R SanLuis
5	7/25/16	5.1	Removed Bio-Rad QC information	A Chini	R SanLuis
5	7/25/16	6.1, 6.2	Replace Bio-Rad QC with STA Coag Controls	A Chini	R SanLuis
5	7/25/16	6.7	Remove QC submission to Bio-Rad	A Chini	R SanLuis
5	7/25/16	8.3	Add QC/Reagent Loading Instructions	A Chini	R SanLuis

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Version	Date	Section	Reason	Reviser	Approval
5	7/25/16	17	Remove Bio-Rad insert	A Chini	R SanLuis
6	5/1/17	Header	Add WAH	L Barrett	R SanLuis
6	5/1/17	4, 6	Remove individual section labeling instructions and add general one	L Barrett	R SanLuis
6	5/1/17	6.3	Remove run QC at beginning of shift	L Barrett	R SanLuis
6	5/1/17	10.5	Review data moved from section 6	L Barrett	R SanLuis
6	5/1/17	15	Update to new standard wording	L Barrett	R SanLuis

19. ADDENDA

A. Instructions for Preparing Collection Tube for Hematocrit >55%
B. Phlebotomist Instructions for Blood Collection

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Appendix A

Instructions for Preparing Collection Tube for Hematocrit >55%

Explanation:

Polycythemia is a disease state in which the proportion of blood volume that is occupied by red blood cells increases - basically when Hematocrit (HCT) is greater than 55%. It can cause prolonged coagulation results.
When a prolonged coagulation result is obtained, check the specimen for a clot first.
If the specimen is not clotted, be sure the specimen is not under-filled or over-filled, then check the HCT result.
If a HCT result of greater than 55% is obtained, immediately notify the doctor or attending nurse and ask for a redraw using a special tube prepared by the lab.

To prepare a special tube in the lab use the following instructions and formula:

The anticoagulant volume in the collection tube must be adjusted to obtain a 9:1 ratio of blood to Sodium Citrate. Under or over-filling of the specially prepared collection tube is not acceptable.
The vacuum in the collection tube will be broken to adjust the volume of collection anticoagulant. Because of this special collection technique, the stability for these whole blood specimens is reduced to four (4) hours after collection.

Formula to calculate the anticoagulant volume is:
Anticoagulant in mL = [(100 – HCT) / (595 – HCT)] x Volume of blood

Example 1: Specimen with a 70% HCT in a 2.7 mL tube:

Patient with HCT of 70%
Using a 2.7 mL tube
Anticoagulant in mL = [(100 – 70) / (595 – 70)] x 2.7 = 0.15 mL or 150 uL
Pipette a 2.7 mL tube in a way to leave only 150 uL of anticoagulant in there.
A 2.7 mL tube contains 0.3mL anticoagulant; therefore remove 0.15mL

Example 2: Specimen with a 70% HCT in a 1.8 mL tube:

Patient with HCT of 70%
Using a 1.8 mL tube
Anticoagulant in mL = [(100 – 70) / (595 – 70)] x 1.8 = 0.1 mL or 100 uL
Pipette a 1.8 mL tube in a way to leave only 100 uL of anticoagulant in there.
A 1.8 mL tube contains 0.2mL anticoagulant; therefore remove 0.1mL

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Example 3: Specimen with a 60% HCT in a 2.7 mL tube:

Patient with HCT of 60%
Using a 2.7 mL tube
Anticoagulant in mL = [(100 – 60) / (595 – 60)] x 2.7 = 0.2 mL or 200 uL
Pipette a 2.7 mL tube in a way to leave only 200 uL of anticoagulant in there.
A 2.7 mL tube contains 0.3mL anticoagulant; therefore remove 0.1mL

Example 4: Specimen with a 60% HCT in a 1.8 mL tube:

Patient with HCT of 60%
Using a 1.8 mL tube
Anticoagulant in mL = [(100 – 60) / (595 – 60)] x 1.8 = 0.13 mL or 130 uL
Pipette a 1.8 mL tube in a way to leave only 130 uL of anticoagulant in there.
A 1.8 mL tube contains 0.2mL anticoagulant; therefore remove 0.07mL

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Appendix B

Phlebotomist Instructions for Blood Collection

The technologist will prepare a special tube in which the anticoagulant has been adjusted, therefore the tube is not vacuumed. The technologist will inform the phlebotomist of the exact amount of blood needed to fill the tube.

Equipment and Supplies

Latex free gloves
Latex free tourniquet
Latex free Band Aid or Tape
Alcohol Prep (70% alcohol)
2x2 sterile gauze
Collection tube
Blood Collection Set 21 or 23 gauge winged set
Blood Transfer Device
3mL syringe
Biohazard bag
Biohazard sharps container
LIS collection list and label/Lab requisition

Collection Steps

1. Introduce yourself to the patient by stating your first and last name.
2. Positively identify the patient according to the SOP 'Patient Identification', Phlebotomy procedure manual.
3. Wash hands. Apply gloves.
4. Explain the procedure to the patient and obtain patient's consent to draw blood.
5. Collect equipment and correct technologist-provided collection tube.
6. Assemble equipment and break needle and syringe seals in the presence of the patient.
7. Apply tourniquet about midway between the elbow and the shoulder 3-4 inches above the venipuncture site). Place patient's arm in a downward position to prevent reflux of 'backflow' of blood from the tube into the venous system. Ask the patient to close hand gently.
8. Palpate/feel for vein locating a vein that will flow fast (reducing the possibility of the blood clotting).
9. Clean the area for venipuncture with a 70% alcohol pad decontaminating the collection site.
10. Allow the area to air-dry completely.
11. Assemble the 21 or 23 gauge winged set to the 3mL syringe. Pull back the plunger to dispel all the air out of the syringe.
12. With the bevel up, align the needle with the vein while holding the skin taut. Insert the needle at a 15-30 degree angle with the skin. Remove your hand from drawing the skin taut. Grasp the syringe and draw back bringing the plunger tip to the exact amount of blood requested by the technologist.
13. Release the tourniquet. Ask the patient to open hand.

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14. Place gauze above the puncture site and remove the needle while simultaneously applying pressure on the puncture sit. Firmly activate needle safety shield, a click must be heard to ensure that the safety shield is secure.
15. Remove 21 or 23 gauge winged set from syringe.
16. Attach the blood-filled syringe to the Blood Transfer Device.
17. Connect the Blood Transfer Device to the un-vacuumed tube, provided by the technologist, and slow and gently fill the collection tube. DO NOT FORCE blood into tube. Pressure can lead to tube explosion and blood exposure.
18. Place the cap on the tube and invert a few times to make sure the anticoagulant is mixed with blood.
19. Dispose of all blood collection equipment into the nearest sharps container. DO NOT disassemble the syringe from the Blood Transfer Device.
20. Dispose of all other used materials in appropriate container and wash hands.
21. Label the sample with the LIS collection label and write the time, date, and your tech code.
22. Transport specimen to the Lab.

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